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U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

APPEAL BRIEF TRANSMITTAL LETTER AND REQUEST FOR EXTENSION OF TIME PURSUANT TO 37 C.F.R. § 1.136(a)	Docket Number: **2653/28**		
Application Number **09/503,852**	Filing Date **February 15, 2000**	Examiner **L. Di Nola-Baron**	Art Unit **1615** / Confirmation No. **5439**
Invention Title **PROTECTION OF THE FEMALE REPRODUCTIVE SYSTEM FROM NATURAL AND ARTIFICIAL INSULTS**		Inventor(s) **TILLY, et al.**	

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By: Carmen H. Apicella

Transmitted herewith is an appeal brief, filed in triplicate, for the above-identified application.

Applicants respectfully request a three-month extension of time in which to respond to the Notice of Appeal filed February 24, 2004, for which a response was due on April 24, 2004. The extended period expires on July 24, 2004.

The Commissioner is hereby authorized to charge payment of the Appeal Brief fee of **$165.00** due under 37 C.F.R. § 1.192(a) and the 37 C.F.R. § 1.136(a) extension fee of **$475.00** to the deposit account of **Kenyon & Kenyon**, deposit account number **11-0600**.

The Commissioner is also authorized to charge payment of additional fees asssociated with this communication or credit any overpayment to deposit account number **11-0600.**

A duplicate copy of this communication is enclosed.

Dated: July 13, 2004

By: Joseph A Coppola

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CUSTOMER NO. 26646

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	JONATHAN L. TILLY et al.
SERIAL NO.	:	09/503,852
FILING DATE	:	February 15, 2000
FOR	:	PROTECTION OF FEMALE REPRODUCTIVE SYSTEM FROM NATURAL AND ARTIFICIAL INSULTS
EXAMINER	:	L. DI NOLA-BARON
GROUP ART UNIT	:	1615
CONFIRMATION NO.	:	5439
CUSTOMER NO.	:	26646

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By: _____
Joseph A. Coppola (Reg. No. 38,416)

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P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Real Party in Interest

The real parties in interest are:

Sloan-Kettering Institute for Cancer Research

1275 York Avenue

New York, NY 10021

Massachusetts General Hospital Corporation

55 Fruit Street

Boston, MA 02114

Sloan-Kettering Institute for Cancer Research and Massachusetts General Hospital are the co-assignees of the entire right, title, and interest in U.S. Patent Application Serial No. 09/503/852.

Related Appeals and Interferences

There are no related appeals or interferences.

Status of Claims

Claims 1, 5-12, 17, 18, 20-23, 32, and 74 are pending.

Claims 3, 4, 13-16, 19, 24-31, 33-73, and 75-80 have been canceled.

Claims 1, 5-12, 17, 18, 20-23, 32, and 74 are being appealed.

Status of Amendments

An Amendment under 37 C.F.R. §116 was filed and was entered for the purposes of appeal. The Amendment under 37 C.F.R. §116 was filed in response to an Office Action, issued December 16, 2003, which contained a Final Rejection. An Advisory Action was issued in response to the Amendment under 37 C.F.R. §116. For the convenience of the Board of Patent Appeals and Interferences, three copies of the Amendment under 37 C.F.R. §116, the Office Action issued December 16, 2003, and the Advisory Action are enclosed.

Three copies of the references Perez et al., Nat, Med. 3:1228-1232 (1997) ("Perez"): U.S. Patent No. 5,712,262 to Spiegel ("Spiegel"); and U.S. Patent No. 5,877,167 to Igarashi et al. ("Igarashi"), discussed herein, are also enclosed.

Summary of the Invention

The claimed invention provides methods of treating a female reproductive system[1] by administering a composition comprising sphingosine-1-phosphate[2] to a female patient in order to inhibit apoptosis[3] induced by a chemotherapeutic drug[4] or radiation.[5] The female patient may be a woman.[6]

[1] Specification, page 5, lines 3-5
[2] Specification, page 6, lines 1-2
[3] Specification, page 9, lines 6-13; Page 17, lines 2-6
[4] Specification, page 5, line 12
[5] Specification, page 5, line 8

All of the appealed claims are directed to treatments that require administration inside the body, either *in vivo* (*i.e.*, on or inside the body) or *ex vivo* (*i.e.*, a composition is initially administered outside the body, *e.g.*, to tissue or cells, but the treated tissue or cells are then returned to the body).[7] The present claims thus stand in sharp contrast to methods that are carried out *in vitro* (*i.e.*, completely outside the body, in isolated tissues or cells which are not returned to the body). Furthermore, the treatments of the present claims are given in response to an insult that occurs *in vivo* (treatment with a chemotherapeutic drug or radiation).

The following illustrates how the limitations of the appealed claims read on the specification.

Claim 1

Claim limitation	Where found in the specification
treating a female reproductive system by administering to a female patient a composition comprising sphingosine-1-phosphate	page 5, lines 3-5; page 6, lines 1-2
in an amount sufficient to inhibit apoptosis induced by an artificial insult	page 9, lines 6-13; page 17, lines 2-6
wherein said administration is *in vivo* or *ex vivo*	page 6, lines 2-3
wherein said artificial insult is a chemotherapeutic drug or radiation	page 5, line 12; page 5, line 8

[6] Specification, page 16, lines 18-21
[7] Specification, page 6, lines 2-3

Claim 5

Claim limitation	Where found in the specification
wherein said chemotherapeutic drug is 5FU, vinblastine, actinomycin D, etoposide, cisplatin, methotrexate, doxorubicin, or a combination thereof	Page 5, lines 14-15

Claim 6

Claim limitation	Where found in the specification
wherein said radiation insult is ionization radiation, x-ray, infrared radiation, ultrasound radiation, heat, or a combination thereof	page 5, lines 17-19

Claim 7

Claim elements	Where found in the specification
wherein said radiation insult comprises an invasive radiation therapy, a non-invasive radiation therapy, or both	page 5, lines 18-19

Claim 8

Claim limitation	Where found in the specification
wherein said female reproductive system comprises ovaries	page 16, lines 15-17

Claim 9

Claim limitation	Where found in the specification
wherein said female reproductive system comprises oocytes	page 16, lines 15-17

Claim 10

Claim limitation	Where found in the specification
wherein said female patient is in a reproductive age	page 5, lines 20-21

Claim 11

Claim limitation	Where found in the specification
wherein said female patient is in a pre-reproductive age	page 5, lines 20-21

Claim 12

Claim limitation	Where found in the specification
wherein said female patient is in a post-reproductive age	page 5, lines 20-21

Claim 17

Claim limitation	Where found in the specification
wherein said composition is administered at least once from about fifteen days to about two days prior to exposure to said insult	page 17, lines 16-18

Claim 18

Claim limitation	Where found in the specification
wherein said composition is administered at about seven days to about two hours prior to exposure to said insult	page 17, lines 16-18

Claim 20

Claim limitation	Where found in the specification

wherein said composition is administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly, intra-uterine, intra-ovarian, rectally, topically, or a combination thereof	page 6, lines 3-5

Claim 21

Claim limitation	Where found in the specification
wherein said artificial insult is a result of a therapy against a disease or a disorder	page 11, lines 11-12

Claim 22

Claim limitation	Where found in the specification
wherein said disease or disorder comprises, cancer, rheumatoid arthritis, angioplasy, or restenosis	page 11, lines 12-13

Claim 23

Claim limitation	Where found in the specification
wherein said cancer comprises; colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chondroma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic	page 11, line 13 to page 12, line 5

carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, acute lymphocytic leukemia and acute myelocytic leukemia, chronic leukemia and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain diseases, or a combination thereof	

Claim 32

Claim limitation	Where found in the specification
wherein said female patient is a woman	page 16, lines 18-21

Claim 74

Claim limitation	Where found in the specification
wherein the artificial insult is a chemotherapeutic drug	page 5, line 12

Issues

The only issue is whether claims 1, 5-12, 17, 18, 20-23, 32, and 74 are obvious under 35 U.S.C. §103(a) over Perez et al., Nat, Med. 3:1228-1232 (1997) ("Perez") in view of U.S.

Patent No. 5,712,262 to Spiegel ("Spiegel") and further in view of U.S. Patent No. 5,877,167 to Igarashi et al. ("Igarashi").

Grouping of Claims

The claims stand or fall together.

Argument

Summary of the Examiner's position

The Examiner concluded that one of ordinary skill in the art would have combined Perez with Spiegel and Igarashi to arrive at the claimed invention. In the December 16, 2003 Office Action (containing the final rejection), the Examiner stated that:

- Perez provides a "strong impetus" to manipulate apoptosis caused by chemical drugs in oocytes, *in vivo*, as a potential means to overcome infertility associated with cancer treatments (page 6, lines 11-13).
- Spiegel teaches that sphingosine-1-phosphate retards apoptosis in degenerative diseases and aging (page 6, lines 16-18).

• Igarashi teaches that sphingosine-1-phosphate can be used to inhibit tumor cell chemoinvasion and provides dosages and routes of administration for the sphingosine-1-phosphate (page 7, lines 3-9).

After characterizing the cited references as summarized above, the Examiner went on to state that one of ordinary skill in the art would have combined Perez, Spiegel, and Igarashi to arrive at the claimed invention with a reasonable expectation of success (page 7, lines 10-20). According to the Examiner, a reasonable expectation of success is found because Spiegel teaches that sphingosine-1-phosphate is effective in treating aging diseases and Igarashi teaches that sphingosine-1-phosphate inhibits tumor cell chemoinvasion. See page 7, lines 16-20:

> Because of the teachings of Spiegel, that sphingosine-1-phosphate is effective in treating aging diseases, and the teachings of Igarashi et al., that sphingosine-1-phosphate inhibits tumor cell chemoinvasion, one of ordinary skill in the art would have had a reasonable expectation that the methods claimed in the instant application would be successful.

The Examiner did not provide an explanation of why methods of treating aging or preventing chemoinvasion would be predictive of success for methods of treating a female reproductive system.

Summary of the Appellants' position

The Appellants submit that the cited references do not make the claims obvious because:

• Perez is directed to the *in vitro* administration of sphingosine-1-phosphate to isolated oocytes to protect against an *in vitro* insult to those isolated oocytes.

- The claims are directed to *in vivo* or *ex vivo* (rather than *in vitro*) administration of sphingosine-1-phosphate to a female patient (not to isolated oocytes) in order to treat the female's reproductive system (not just isolated oocytes).
- Perez contains explicit statements of doubt as to whether Perez's *in vitro* results with isolated oocytes can be successfully extrapolated to methods of treating the female reproductive system (as opposed to isolated oocytes).
- Spiegel and Igarashi have absolutely nothing to do with oocytes or female reproductive systems and thus should not be combined with Perez. Even if so combined, Spiegel and Igarashi cannot overcome the statements of doubt in Perez as to treating female reproductive systems since Spiegel and Igarashi have nothing to do with female reproductive systems.

Detailed explanation of the Appellants' position

The cited references

Perez discloses studies in which sphingosine-1-phosphate was administered *in vitro* to isolated oocytes that were also exposed *in vitro* to doxorubicin. See page 1228, left column, second line from bottom, where Perez states that the oocytes that were studied were "harvested [*i.e.*, isolated] from superovulated adult female mice" and "maintained in human tubal fluid medium under standard *in vitro* conditions." See page 1229, Figure 2, and the discussion of Figure 2 in the left column, where Perez states that sphingosine-1-phosphate was administered to the isolated oocytes. Perez did not disclose studies in which sphingosine-1-phosphate was administered either *in vivo* or *ex vivo*. In Perez, the oocytes were never returned to the body, but

were merely observed *in vitro*. Moreover, the insult (doxorubicin exposure) to the oocytes that were administered sphingosine1-phosphate occurred *in vitro*.

Spiegel is directed to "methods of retarding apoptosis in degenerative diseases, including neurodegenerative diseases and aging, ..." (December 16, 2003 Office Action, page 6, lines 16-17). Speigel disclosed the use of sphingosine-1-phosphate for this purpose.

Igarashi is directed to "methods of inhibiting tumor cell chemoinvasion." (December 16, 2003 Office Action, page 7, line 3). Igarashi disclosed the use of sphingosine-1-phosphate for this purpose.

Spiegel and Igarashi do not discuss oocytes or female reproductive systems.

Differences between the claims and the cited references

The present claims are directed to methods of "treating a female reproductive system by administering to a female patient ... sphingosine-1-phosphate ... wherein said administration is *in vivo* or *ex vivo* ..." These methods do not encompass treating isolated oocytes *in vitro*, without returning the oocytes to the body. All of these methods require the *in vivo* or *ex vivo* use of sphingosine-1-phosphate to treat the female patient (as opposed to merely isolated cells). Moreover, the present claims are directed to treatments that are given in response to insults that occur *in vivo* rather than *in vitro*.

Perez is directed only to the *in vitro* use of sphingsine-1-phosphate (*i.e.*, administered to isolated oocytes) and not its *in vivo* or *ex vivo* use. The use of sphigosine-1-phosphate in Perez is

limited to use in conjunction with *in vitro* insults. Spiegel and Igarashi are not directed to treating the female reproductive system in any manner.

The rejection for obviousness

The Examiner concluded that one of ordinary skill in the art would have combined Perez with Spiegel and Igarashi to arrive at the claimed invention. The reasons for this conclusion are given in the December 16, 2003 Office Action at page 7, line 11 to page 8, line 2:

> Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to apply the teachings of Perez et al. and Spiegel to device [sic] methods of protecting the female reproductive system, reviving the ovarian function or ameliorating menopausal syndromes in women, comprising administering SPP [sphingosine-1-phosphate] compositions, and determining the mode and dosage of administration according to the teachings of Igarashi et al. The expected result would have been successful methods of treatment. Because of the teachings of Spiegel, that sphingosine-1-phosphate is effective in treating aging diseases, and the teachings of Igarashi et al., that sphingosine-1-phosphate inhibits tumor cell chemoinvasion, one of ordinary skill in the art would have had a reasonable expectation that the methods claimed in the instant application would be successful. Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Why the rejection should be withdrawn

The cited references do not provide a reasonable expectation of success for the claimed invention

The Applicants submit that Perez, Spiegel, and Igarashi, in any combination, do not provide a reasonable expectation of success for the claimed invention (or even a motivation to try).

It is well settled that a finding of obviousness requires that the cited references provide a reasonable expectation of success for the claimed invention. It is not sufficient that the references make it obvious to try to make the claimed invention. See, *e.g.*, In re Vaeck, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991), where the Federal Circuit said:

> [A] proper analysis under §103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.*, 837 F2d 469, 473, 5 U.S.P.Q. 2d 1529, 1531 (Fed. Cir. 1988). [emphasis added]

Perez alone cannot provide a reasonable expectation of success for the claimed invention because:

- Perez is directed to an *in vitro* method (administering sphingosine-1-phosphate to isolated oocytes) to protect against an *in vitro* insult (administering doxorubicin to isolated oocytes).
- The present claims do not encompass *in vitro* methods to protect against *in vitro* insults.
- Perez contains explicit statements of doubt as to whether Perez's *in vitro* results with isolated oocytes can be extrapolated to *in vivo* treatment of the female reproductive system as in the present claims. For example, Perez made it clear that *in vitro* effects are not predictive of *in vivo* successes such as preserving ovarian function. See page 1231, sentence bridging left and right columns:

> Despite the significant advances made by this study in defining the biochemical and genetic pathways involved in oocyte destruction following exposure to anticancer drugs, future long-term studies are required to confirm that inhibiting germ cell apoptosis will preserve ovarian function. [emphasis added]

Perez also taught that *in vivo* treatments of the female reproductive system (*e.g.*, preserving its fertility) require an effect not just on oocytes, but also on the follicles that support oocytes. See page 1230, lines 6-7: "[F]ertility preservation would require maintenance of the entire follicle and not solely the oocyte." Perez, page 1230, col. 1, lines 6-7. Perez contains no demonstration of the effects of sphingosine-1-phosphate on follicles and thus cannot provide a reasonable expectation of success for treatments that depend on effects on follicles.

The follicle is a structure found in ovaries that is formed by the physical interaction of oocytes and granulosa cells and is necessary for the growth, maturation, and survival of oocytes. Oocytes degenerate and die fairly quickly if not supported by the somatic cells found within follicles. For example, oocytes can survive for months (in mice) to years (in humans) when enclosed within follicles, but only for 24-48 hours once removed and placed in vitro. The *in vitro* results of Perez completely ignore the possibility of damage to other (non-oocyte) cells, such as either follicular granulosa cells or microvascular endothelial cells (blood supply), playing a role in oocyte depletion and ovarian failure caused by anti-cancer treatments.

It is clear that providing protection against radiation damage *in vivo* requires protecting granulosa cells as well as oocytes. Therefore, studies such as Perez, which shed no light on the ability of sphingosine-1-phosphate to protect granulosa cells from radiation damage, cannot provide a reasonable expectation of success for the practice of the claimed invention.

Moreover, the work described in Perez was conducted solely *in vitro* using fully mature (metaphase II) oocytes obtained after superovulating female mice with exogenous gonadotropins (see page 1231, right column, under "Methods"). These fully mature oocytes are not comparable in terms of radiation sensitivity to the immature (germinal vesicle-stage)

oocytes contained within the resting (primordial) and early growing (primary) follicles found *in vivo*. It is these immature oocytes that are the target population of the claimed invention. This target population of oocytes is not comparable to the oocytes of Perez because immature oocytes are much more sensitive to, and thus preferentially destroyed by, radiation *in vivo*.

With respect to what implications Perez's *in vitro* results have for *in vivo* and *ex vivo* methods such as those presently claimed, the Examiner stated: "[T]he data from the study [*i.e.*, from Perez] provide a strong impetus to manipulate apoptosis caused by chemical drugs in oocytes, in vivo, as a potential means to overcome infertility associated with cancer treatment." [first underlining added] (December 16, 2003 Office Action, page 6, lines 11-13)

The Applicants believe that the statement quoted above demonstrates at most that Perez makes it obvious to try the claimed invention. This is supported by the Examiner's use of the word "impetus" which speaks to motivation or suggestion rather than expectation of success.

The Examiner did not argue that Perez alone provided a reasonable expectation of success. The Examiner instead argued that it was the combination of Spiegel and Igarashi with Perez that provided a reasonable expectation of success. See the December 16, 2003 Office Action, at page 7, lines 16-20:

> Because of the teachings of Spiegel, that sphingosine-1-phosphate is effective in treating aging diseases, and the teachings of Igarashi et al., that sphingosine-1-phosphate inhibits tumor cell chemoinvasion, one of ordinary skill in the art would have had a reasonable expectation that the methods claimed in the instant application would be successful.

In other words, the Examiner relied on references directed to neurodegenerative aging diseases and the chemoinvasion of tumor cells in order to provide a reasonable expectation of success for an invention directed to the treatment of the female reproductive system. These three

types of health problems have no obvious connection and the Examiner has not provided an explanation of why they might be connected. The Applicants do not understand how the expressions of doubt as to reasonable expectation of success in Perez quoted above can be negated by two references that are directed to entirely different health problems from those of both Perez and the present claims.

The cited references should not be combined

That the Examiner could not explain how Spiegel and Igarashi might provide a reasonable expectation of success is not surprising when one considers the content of the references. Spiegel and Igarashi cannot bridge the gap between Perez's *in vitro* results in oocytes and the Applicants' invention directed to the female reproductive system because Spiegel and Igarashi have absolutely nothing to do with oocytes or female reproduction.

Spiegel is directed to "methods of retarding apoptosis in degenerative diseases, including neurodegenerative diseases and aging" (December 16, 2003 Office Action, page 6, lines 16-17). Igarashi is directed to "methods of inhibiting tumor cell chemoinvasion." (December 16, 2003 Office Action, page 7, line 3). The Examiner never contended that Spiegel or Igarashi discuss oocytes or female reproduction.

Spiegel and Igarashi are not directed to the field of the Applicants' invention, female reproductive systems. Nor are Spiegel and Igarashi directed to the particular problems solved by the present claims: treating a female reproductive system by inhibiting apoptosis caused by a chemotherapeutic drug or radiation.

References that are directed neither to the field of the applicant's invention or to the particular problem with which the applicant is concerned may not be used to support an obviousness rejection. See, *e.g.*, In re Oetiker, 977 F.2d 1443, 1447, 24 USPQ2d 1443, 1445 (Fed. Cir. 1992):

> In order to rely on a reference as a basis for rejection of the applicant's invention, the reference must either be in the field of the applicant's endeavor, or, if not, then be reasonably pertinent to the particular problem with which the inventor was concerned.

Since Spiegel and Igarashi are directed neither to the field of the Applicants' invention or to the particular problem with which the Applicants were concerned, Spiegel and Igaraashi should not have been combined with Perez.

In view of the above, the Appellants submit that it has been demonstrated that claims 1, 5-12, 17, 18, 20-23, 32, and 74 are not obvious under 35 U.S.C. §103(a) over Perez, Spiegel, and Igarashi.

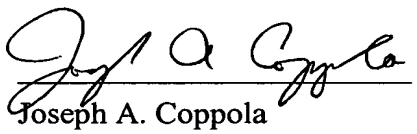
CONCLUSION

For the reasons discussed above, the Appellants respectfully request that the Board of Patent Appeals and Interferences reverse the rejection of claims 1, 5-12, 17, 18, 20-23, 32, and 74 under 35 U.S.C. §103(a).

Respectfully submitted,

KENYON & KENYON

Dated: July 13, 2004

By: 

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APPENDIX

1. A method of treating a female reproductive system by administering to a female patient a composition comprising sphingosine-1-phosphate in an amount sufficient to inhibit apoptosis induced by an artificial insult, wherein said administration is *in vivo* or *ex vivo*, and wherein said artificial insult is a chemotherapeutic drug or radiation.

5. The method of claim 1, wherein said chemotherapeutic drug is 5FU, vinblastine, actinomycin D, etoposide, cisplatin, methotrexate, doxorubicin, or a combination thereof.

6. The method of claim 1, wherein said radiation insult is ionization radiation, x-ray, infrared radiation, ultrasound radiation, heat, or a combination thereof.

7. The method of claim 1, wherein said radiation insult comprises an invasive radiation therapy, a non-invasive radiation therapy, or both.

8. The method of claim 1, wherein said female reproductive system comprises ovaries.

9. The method of claim 1, wherein said female reproductive system comprises oocytes.

10. The method of claim 1, wherein said female patient is in a reproductive age.

11. The method of claim 1, wherein said female patient is in a pre-reproductive age.

12. The method of claim 1, wherein said female patient is in a post-reproductive age.

17. The method of claim 1, wherein said composition is administered at least once from about fifteen days to about two days prior to exposure to said insult.

18. The method of claim 17, wherein said composition is administered at about seven days to about two hours prior to exposure to said insult.

20. The method of claim 1, wherein said composition is administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly, intra-uterine, intra-ovarian, rectally, topically, or a combination thereof.

21. The method of claim 1, wherein said artificial insult is a result of a therapy against a disease or a disorder.

22. The method of claim 21, wherein said disease or disorder comprises, cancer, rheumatoid arthritis, angioplasty, or restenosis.

23. The method of claim 22, wherein said cancer comprises; colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, fibrosarcoma, myxosarcoma, liposarcoma,

chondrosarcoma, osteogenic sarcoma, chondroma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, acute lymphocytic leukemia and acute myelocytic leukemia, chronic leukemia and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain diseases, or a combination thereof.

32. The method of claim 1, wherein said female patient is a woman.

74. The method of claim 1, wherein the artificial insult is a chemotherapeutic drug.

Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction

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Female sterility resulting from oocyte destruction is an unfortunate, and in many cases inevitable, consequence of chemotherapy. We show that unfertilized mouse oocytes exposed to therapeutic levels of the antitumor drug, doxorubicin (DXR), undergo apoptosis; however, fertilized oocytes do not initiate apoptosis, but enter cell-cycle arrest, when treated with DXR. Apoptosis induced by DXR in oocytes is blocked by sphingosine-1-phosphate, an inhibitor of ceramide-promoted cell death. Oocytes from Bax-deficient, but not p53-null, female mice display complete resistance to DXR-induced apoptosis *in vivo* and *in vitro*. Pretreatment of oocytes with a specific peptide inhibitor of caspases also abrogates the apoptotic response to DXR. These findings indicate that oocyte destruction caused by chemotherapy can be prevented by manipulation of apoptosis-associated signaling pathways.

Conventional cancer therapies kill normal cells in addition to the tumor cells targeted for destruction[1–4]. One of the most sensitive of these noncancerous cell types is the ovarian germ cell. The female gonads house a finite number of germ cells (haploid oocytes) enclosed within primordial follicles that serve as the stockpile of eggs released at ovulation each menstrual cycle for potential fertilization[5]. Once depleted, the ovarian germ cell pool cannot be replenished. Thus, exposure of women to a wide spectrum of agents that damage the ovary generally leads to irreversible sterility[1–4]. Although sensitivity of oocytes to chemo- and radiotherapy is well-documented[1–4], nothing is known regarding the mechanisms responsible for female germ cell destruction. Because of this potential for destruction, coupled with the inefficiency of human oocyte cryopreservation[6], women of reproductive capacity have little hope of conception following many chemotherapeutic regimens.

Apoptotic cell death plays a fundamental role in normal germ cell endowment and follicular dynamics in the ovary[7–9], and cell fate in this organ is likely dependent on the actions of several proteins recently identified as key determinants of cell survival[9–12]. Among these are p53 (ref. 13, 14) and members of the *bcl-2* (ref. 15–18) and *CASP* (ref. 19) (*ced-3/Ice*)[20,21] gene families. In addition, ceramide, a recently identified lipid second messenger associated with cell death signaling[22,23], has been implicated in the induction of apoptosis in the ovary[24–26]. Therefore, we determined if the widely used chemotherapeutic drug, doxorubicin (DXR; 14-hydroxydaunomycin or adriamycin), leads to sterility in females by triggering apoptosis in oocytes following activation of one or more of these cell death effector pathways.

Doxorubicin induces oocyte apoptosis

Mature oocytes harvested from superovulated adult female mice were maintained in human tubal fluid medium under standard *in vitro* conditions. Based on the range of therapeutic plasma levels (100–400 nM) observed in patients treated with this class of drugs[27,28], as well as on data derived from preliminary experiments to identify a median effective concentration of DXR for apoptosis induction in isolated oocytes (data not shown), a dose of 200 nM DXR was selected for use. A significant proportion of the oocytes cultured for 24 hours with DXR exhibited condensation, budding and cellular fragmentation indicative of apoptosis (Fig. 1, *a–c*). Fluorescence microscopy of DXR-treated oocytes following Hoechst 33342-staining revealed segregation of genomic DNA into multiple apoptotic bodies (Fig. 1*d*). The percentage of untreated oocytes displaying the apoptotic morphology following 24 hours of culture was negligible (<3%; Fig. 2), whereas the level of apoptosis in oocytes treated with DXR (>60%; Fig. 2) was comparable to that observed in P388 murine leukemia cells exposed to similar concentrations of the DXR-like drug, daunorubicin (DNR; daunomycin), for 24 hours[29].

To determine if the apoptotic response to DXR is specific for the haploid oocyte, zygotes (fertilized oocytes) collected from superovulated female mice 16 hours after mating were subjected to the same experimental manipulation. Once released from meiotic ar-

Table 1 Cell-cycle arrest in fertilized mouse oocytes treated with DXR

	1-cell to 2-cell	2-cell to 4-cell	4-cell to morula
Control	83 ± 6% (185)	88 ± 5% (129)	93 ± 3% (96)
DXR	<2%* (66)	0% (57)	<2%* (59)

Early embryonic development was assessed by monitoring the percentage of embryos progressing from the 1-cell to 2-cell stage, 2-cell to 4-cell stage, or 4-cell stage to morula in the absence (control) or presence of 200 nM DXR. Note that once fertilization of the haploid oocyte occurs, DXR no longer elicits apoptosis (see Fig. 3) but causes cell-cycle arrest. The total number of embryos evaluated under each experimental condition are given in parentheses.
*One embryo out of the total number analyzed progressed to the next indicated stage of development.

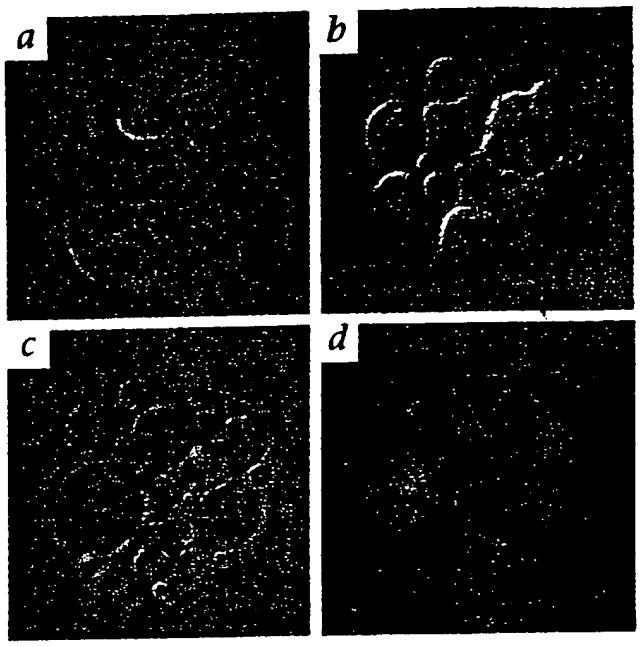

Fig. 1 Apoptotic features in DXR-treated mouse oocytes. Morphology (*a–c*) and DNA fluorescence microscopy (*d*) of isolated mouse oocytes following *in vitro* treatment with vehicle (*a*) or with 200 nM DXR (*b–d*) for 24 h. Mature oocytes (*a*, arrow indicates extruded polar body) exhibited cellular condensation, budding and fragmentation indicative of apoptosis (*b* and *c*) following exposure to therapeutic levels of DXR. Fluorescence microscopy following Hoechst 33342-staining of the DXR-treated oocyte depicted in *c* revealed segregation of DNA into several of the apoptotic bodies (*d*, DNA indicated by arrows).

rest by fertilization, the now diploid zygotes did not undergo apoptosis following treatment with DXR (Fig. 3 and Table 1). In fact, early embryonic progression, *in vitro*, of successive mitotic divisions of the fertilized oocyte (1-cell to 2-cell, 2-cell to 4-cell, 4-cell to morula) was almost completely inhibited by the presence of DXR (Fig. 3 and Table 1). Development of untreated zygotes proceeded normally, with the majority of the fertilized oocytes reaching the morula stage within 72 hours *in vitro* (Fig. 3 and Table 1). Consequently, the proapoptotic action of DXR in female germ cells is specific for the haploid oocyte, whereas once fertilization takes place the actions of DXR become manifested as cell-cycle arrest.

Ceramide mediates DXR-induced oocyte death

One of the immediate signals for cell death generated in tumor cells by treatment with DNR is believed to be the lipid second messenger, ceramide[29,30]. Recent investigations have shown that the production of ceramide, and the ensuing onset of apoptosis, in P388 cells cultured in the presence of DNR is prevented by pretreatment with fumonisin-B1 (ref. 29), a fungal toxin that specifically represses the activity of ceramide synthase[31]. However, multiple, cell type-specific mechanisms exist for ceramide generation following exposure to these drugs[30]. In haploid oocytes, the lethality of DXR was not blocked by pretreatment with fumonisin-B1 (Fig. 2). In contrast, pretreatment of oocytes with sphingosine-1-phosphate (SP), an endogenous downstream inhibitor of ceramide-promoted intracellular signaling[32], maintained oocyte survival in DXR cultures (Fig. 2). These findings are consistent with the proposal that ceramide may serve as an early signal for apoptosis induction in oocytes triggered by DXR. The ability of SP to prevent DXR-induced apoptosis in germ cells also argues for the existence of a discrete intracellular signaling pathway(s) activated in oocytes by chemotherapeutic drugs, such as that involving stress kinases known to be targeted by ceramide and SP in cells[32].

Bax is required for DXR-initiated oocyte apoptosis

The downstream effectors that alter cell death potential and/or carry out the order for cellular suicide are numerous, and include members of the *bcl-2* gene family[10,12]. Mice harboring a targeted disruption of the *bax* death-susceptibility gene exhibit a number of phenotypic abnormalities in the ovary, including apparent defects in follicular cell death during atresia[17]. Conversely, gene targeting-mediated ablation of functional Bcl-2, a heterodimeric partner for Bax that promotes cellular survival[12,33], causes a significant reduction in the number of primordial follicles in the postnatal mouse ovary[16]. Collectively, these observations implicate the ratio of Bcl-2:Bax as a fundamental determinant of ovarian cell fate under normal conditions[8,9]. Consistent with the hypothesis that DXR causes germ cell destruction via activation of apoptotic cell death pathways, oocytes collected from superovulated, Bax-deficient female mice displayed almost complete resistance to DXR-induced apoptosis (Fig. 4). By comparison, oocytes harvested in parallel from wild-type (+/+) or heterozygote (+/-) sister littermates exhibited the expected rates of apoptosis following DXR treatment *in vitro* (Fig. 4).

The resistance of Bax-deficient oocytes to chemotherapy was further tested by intraperitoneal injections of wild-type and Bax-null female mice with DXR followed by morphometric and morphologic evaluations of the ovaries. Wild-type female mice injected with DXR exhibited significant losses in primordial follicles over the 3-week treatment period, as reflected by a decrease in follicle number and the presence of numerous primordial follicle-like structures lacking oocytes (Fig. 5, *a–c*). However, Bax-null female mice did not exhibit oocyte destruction nor a loss of

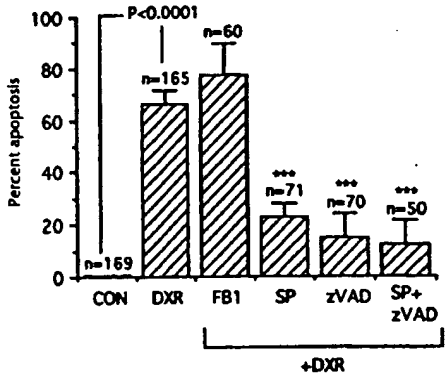

Fig. 2 Inhibition of DXR-induced apoptosis in mouse oocytes by sphingosine-1-phosphate or zVAD-FMK. Pretreatment of oocytes with an inhibitor of ceramide-promoted signaling events, sphingosine-1-phosphate (SP, 10 μM), or with a caspase inhibitor, zVAD-FMK (zVAD, 10 μM), attenuated DXR-induced apoptosis. By comparison, pretreatment with the ceramide synthase inhibitor, fumonisin-B1 (FB1, 100 μM), did not abrogate the apoptotic response of oocytes to 200 nM DXR. In all cases, the pretreatments (FB1, SP, zVAD, SP+zVAD) were added to oocyte cultures 30 min before the addition of 200 nM DXR, and cultures were then continued for 23.5 h. In the absence of DXR, none of the inhibitors tested altered oocyte survival (data not shown). The numbers of oocytes used under each experimental condition (n) are provided above the respective bar (means ± s.e.m., ***$P<0.01$).

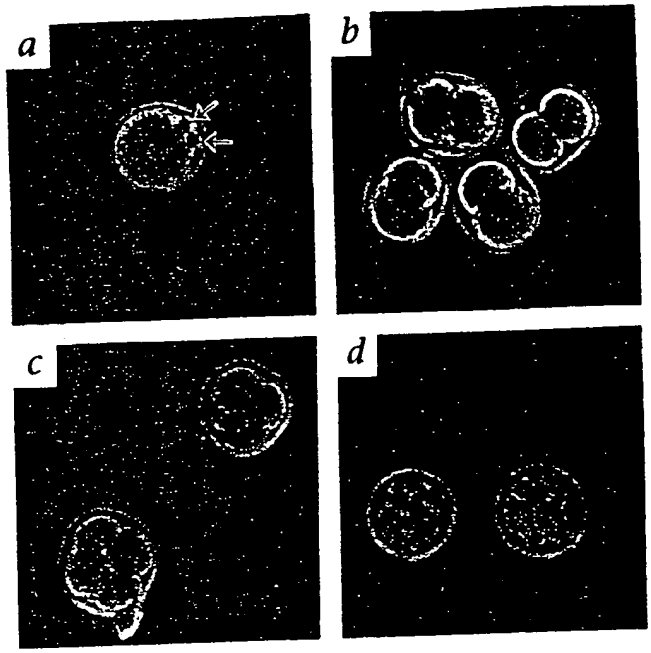

Fig. 3 Resistance of fertilized mouse oocytes to DXR-induced apoptosis. Once fertilized (confirmed by the presence of two polar bodies, indicated by arrows in *a*), one-cell zygotes became growth arrested in the presence of 200 nM DXR as progression to the next stage of development (two-cell stage, *b*) was blocked. Similarly, progression of embryos from the two-cell stage (*b*) to the four-cell stage (*c*), as well as from the four-cell stage (*c*) to the morula stage (*d*), was prevented by treatment of each stage embryo with 200 nM DXR (Table 1). However, not one embryo exposed to DXR exhibited evidence of apoptosis (*a–d*). Embryonic development in control cultures proceeded normally with the majority of embryos reaching the morula stage within 72 h (Table 1).

primordial follicles in response to DXR (Fig. 5, *d* and *e*), collectively revealing a fundamental role for Bax in oocyte death caused by chemotherapy *in vivo* and *in vitro*. The *in vivo* observations also demonstrate that both germ cells and follicular somatic (granulosa) cells are protected in Bax-deficient mice treated with DXR, an important feature since fertility preservation would require maintenance of the entire follicle and not solely the oocyte.

P53 is not required for DXR-induced oocyte destruction

In some cell systems, Bax appears to function as a key downstream effector of apoptosis triggered by p53. For instance, Bax deficiency accelerates tumorigenesis *in vivo* in a mouse brain model dependent on p53 loss-of-function for tumor growth[34], and p53-dependent apoptosis in E1A-transfected mouse embryonic fibroblasts is partially reversed by Bax-deficiency[35]. However, a p53-dependent, Bax-independent pathway leading to cell death must also exist since normal and Bax-deficient mouse thymocytes undergo comparable rates of p53-dependent apoptosis in response to irradiation[17]. Since p53 is highly expressed in oocytes[36] [see also, A. Jurisicova *et al.*, *J. Soc. Gynecol. Invest.* Suppl. 4, 93A (Abstr.); 1997] and is thought to play a prominent role in apoptosis induction in tumor cells treated with chemotherapeutic drugs[37–39], we next assessed the requirement for p53 in mediating DXR-induced apoptosis in female germ cells. In contrast to the striking protection conveyed by Bax-deficiency, oocytes harvested from mice with a targeted disruption in the *p53* gene exhibited levels of apoptosis following DXR treatment that were comparable to those observed in wild-type oocytes (Fig. 4). These findings indicate that DXR can activate apoptosis in oocytes via a Bax-dependent, but p53-independent, mechanism. Although the existence of p53-independent pathways of apoptosis induction in some normal and tumor cells have been reported[34,39], the requirement for Bax in these models has not yet been directly tested. Moreover, the lack of requirement for p53 in chemotherapy-induced germ cell death indicates that the involvement of Bax in oocyte apoptosis is not dependent on p53-mediated transcriptional events at the level of the *bax* gene promoter[40].

DXR requires caspases to promote oocyte death

Another cohort of downstream cell death effector molecules important for apoptosis execution are proteases encoded by the *CASP* (cysteine aspartic acid-specific protease) gene family[19,41]. Pretreatment of oocytes with the cell-permeable and specific peptide inhibitor of caspases, zVAD-FMK (ref. 41–43), markedly attenuated their apoptotic response to DXR (Fig. 2). The effects of zVAD-FMK in our experiments are likely specific for inhibition of this class of proteases since oocytes collected from genetically-manipulated female mice that do not express functional caspase-2 (Ich-1) exhibit the same degree of resistance to DXR-induced apoptosis [L. Bergeron *et al.*, Proc. 2nd Keystone Symp. on Apoptosis and Programmed Cell Death, p. 31 (Abstr.); 1997; manuscript submitted] as that observed herein with wild-type oocytes treated with the caspase inhibitor (Fig. 2). Taken with the requirement for Bax in chemotherapy-induced oocyte apoptosis, these findings are consistent with the hypothesized "ordering" of the cell death pathway with caspases acting downstream of Bcl-2 family members[44]. However, our findings with oocytes differ from a recent report that death of Jurkat cells induced

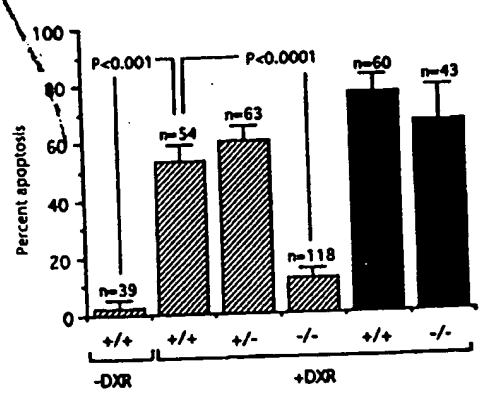

Fig. 4 Bax-deficient, but not p53-null, mouse oocytes are protected from DXR-induced apoptosis. Hatched bars depict data obtained with Bax-deficient and wild-type female mice. Culture of oocytes obtained from wild-type (+/+) or heterozygote (+/-) sister littermates in the presence of 200 nM DXR for 24 h resulted in the expected levels of apoptosis (compare with Fig. 2), whereas oocytes harvested from Bax-deficient sister littermates (-/-) were almost completely resistant to DXR-induced apoptosis. Black bars depict data obtained following a similar analysis of oocytes obtained from wild-type and p53-deficient female mice. In contrast to Bax-null mice, the extent of DXR-induced death in oocytes of p53-deficient mice was not different that that observed in the wild-type controls. The numbers of oocytes used for each data point (*n*), along with statistical comparisons, are provided above the respective bar (means ± s.e.m.).

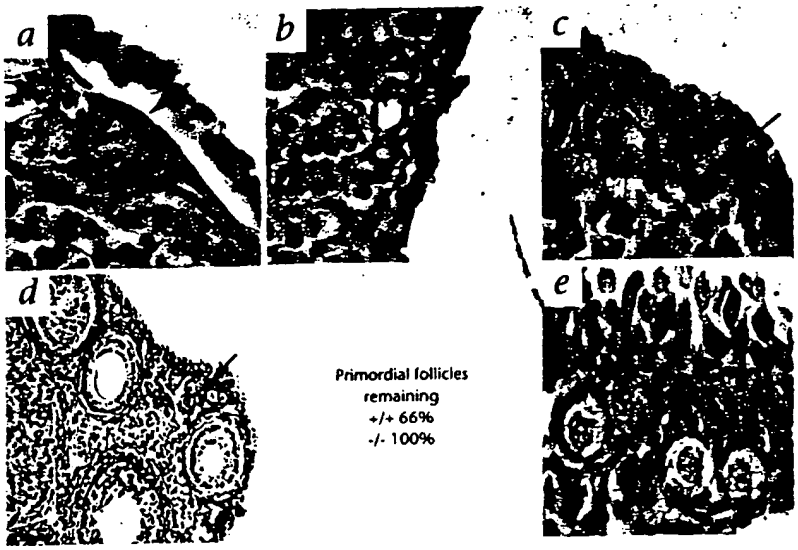

Fig. 5 Resistance of Bax-null female mice to DXR-induced oocyte destruction *in vivo*. Wild-type and Bax-deficient female mice were given two intraperitoneal injections of vehicle or DXR (10 mg/kg of body weight) 1 week apart, starting at 8 weeks of age post-partum. One week following the final injection, ovaries were collected for morphologic and morphometric evaluations. Representative photomicrographs of the histology of wild-type (+/+; *a–c*) and Bax-null (-/-; *d* and *e*) ovaries post-DXR treatment are shown. In *b*, a primordial follicle-like structure (for example, lacking an oocyte) in a DXR-treated wild-type ovary is indicated by the arrow, whereas *c* depicts oocytes in the process of degeneration. Oocyte destruction was also noted in primordial follicles undergoing transition to the primary stage (*a*, indicated by the arrow). By comparison, primordial follicles in Bax-null females were unaffected by DXR treatment, as revealed by histologic appearance (*d* and *e*; oocyte-containing primordial follicles are indicated by arrows) and the number of oocyte-containing primordial follicles remaining post-DXR treatment (expressed as a percent of those follicles present in vehicle-treated control ovaries for each group).

by overexpression of *bax* following gene transfer *in vitro* may not require caspases (at least those that can be inhibited by zVAD-FMK)[45], although caspases are apparently involved in Bax-induced apoptosis in other somatic cell types[46].

Discussion

Elucidation of specific events triggered in oocytes that lead to apoptosis following DXR exposure is the first demonstration of the functional existence of such pathways in meiotic cells. Since unfertilized oocytes are haploid, the lack of requirement for p53 in this paradigm of cell death may be due, at least in part, to the fact that this protein does not function in cells completely incapable of mitotic division as compared with the fundamental role of p53 in mitotically competent, diploid somatic cells[38,39]. Moreover, the metaphase II oocytes used in our *in vitro* studies do not possess a defined nucleus. The genetic material of the mature female gamete is "free-floating" within the ooplasm awaiting contribution of similar genetic information from the male germ cell following fertilization before a nucleus is re-formed and embryogenesis is initiated. Although many efforts have been made to dissect the role of nuclear versus cytoplasmic events in cell death committal[47,48], these previous studies required physical separation of the nucleus from the remaining cellular components (cytoplasts or cell extracts) before experimental manipulation. Since mature oocytes were clearly capable of undergoing apoptotic cell death, these data provide the first documentation in an undisturbed, physiologically intact cell system that a nucleus is not required for apoptosis driven by specific intracellular signaling events.

In conclusion, this study shows that female germ cells exposed to a widely used chemotherapeutic drug initiate apoptosis as a consequence of the activation of several death effector signaling pathways probably involving ceramide, Bax and caspases, but not p53. At present, it remains to be established whether these signals act in parallel pathways or, if in a cascade, how upstream messengers such as ceramide may communicate with more distal effectors including Bax and caspases. Despite the significant advances made by this study in defining the biochemical and genetic pathways involved in oocyte destruction following exposure to anticancer drugs, future long-term studies are required to confirm that inhibiting germ cell apoptosis will preserve ovarian function. Nevertheless, these data provide a strong impetus for our current efforts to manipulate death effector pathways in oocytes, *in vivo*, as a potential means to overcome infertility associated with cancer treatment.

Methods

***In vitro* oocyte cultures.** All experiments involving animals described herein were approved by, and performed in strict accordance with the guidelines of, the Massachusetts General Hospital Institutional Animal Care and Use Committee. Except for those experiments involving Bax- or p53-null oocytes (see below), female B6C3F1 mice (43 days of age post-partum; Charles River Laboratories, Wilmington, MA) were superovulated with 10 IU of equine chorionic gonadotropin (eCG or PMSG; Professional Compounding Centers of America, Houston, TX) followed by 10 IU of human chorionic gonadotropin (hCG; Serono Laboratories, Norwell, MA) 48 h later. Mature oocytes were collected from the oviducts 16 h after hCG injection. Cumulus-enclosed oocytes were denuded by a 1-min incubation in 80 IU/ml of hyaluronidase (Sigma Chemical Co., St. Louis, MO), followed by three washes with culture medium. The medium used for all culture experiments was human tubal fluid (Irvine Scientific, Santa Ana, CA) supplemented with 0.5% bovine serum albumin (BSA, Fraction V; Gibco-BRL Life Technologies, Grand Island, NY). Oocytes were cultured in 0.1 ml drops of culture medium (8–10 oocytes/drop) under paraffin oil, and incubated without or with DXR (200 nM; Sigma) and/or fumonisin-B1 (Sigma), sphingosine-1-phosphate (Biomol, Plymouth Meeting, PA) or benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-FMK; Enzyme Systems Products, Dublin, CA) for 24 h at 37 C in a humidified atmosphere of 5% CO_2-95% air. At the end of the incubation period, oocytes were fixed, stained with Hoechst 33342 (Sigma) and checked microscopically for morphological changes characteristic of apoptosis (condensation, budding, cellular fragmentation, and chromatin segregation into apoptotic bodies). The percentage of oocytes that underwent apoptosis out of the total number of oocytes cultured per drop in each experiment was then determined, and all experiments were independently repeated 4–10 times with different mice.

***In vitro* embryo cultures.** Adult female B6C3F1 mice were superovulated with eCG followed by hCG treatment (see above) and placed with fertile males immediately after hCG injection. Sixteen hours after mating, one-cell embryos (confirmed by the presence of two polar bodies) were harvested from the ampullae and denuded of cumulus cells by a 1-min hyaluronidase treatment. Embryos were then maintained *in vitro* in HTF supplemented with 0.5% BSA in the absence or presence of 200 nM DXR. Under our *in vitro* conditions, one-cell embryos progress to the morula stage of development within 72 h (refer to *In vitro* oocyte cultures above for details of methodology and culture conditions).

Bax-null mice. For the *in vitro* experiments, mature oocytes were harvested from wild-type and Bax-null[17] adult female C57BL6 mice (at approximately 6 weeks of age) using the gonadotropin superovulation regimen described above. Following hyaluronidase removal of cumulus cells, oocytes were incubated for 24 h without or with 200 nM DXR, after which the occurrence of apoptosis was assessed as detailed under *In vitro* oocyte cultures. For the *in vivo* experiments, age-matched adult wild-type and Bax-null[17] female mice were given two intraperitoneal injections of DXR (10 mg/kg of body weight) 1 week apart, starting at approximately 8 weeks of age post partum. One week following the second injection, ovaries were collected, fixed, embedded in paraffin, serial-sectioned, and stained with hematoxylin/picric methyl blue. Follicular morphology and numbers of immature (primordial) follicles present in each ovary were then assessed as detailed previously[14].

p53-null mice. Mature oocytes were collected from adult wild-type and p53-null (Taconic Farms, Germantown, NY) C57BL6 female mice by superovulation, and incubated without and with 200 nM DXR for 24 h. Following culture, the occurrence of apoptosis was assessed as described above (see *In vitro* oocyte cultures).

Data presentation and statistical analysis. The combined data from the replicate experiments were subjected to a one-way analysis of variance followed by Scheffé's *F*-test, and statistical significance was assigned at $P < 0.05$. Graphs represent the means (± s.e.m. of combined data from the replicate experiments, whereas representative photomicrographs are presented for the oocyte morphology or ovarian histology.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/503,852	02/15/2000	Jonathan L. Tilly	2653/28	5439

23838 7590 03/22/2004

KENYON & KENYON
1500 K STREET, N.W., SUITE 700
WASHINGTON, DC 20005

EXAMINER	
DI NOLA BARON, LILIANA	

ART UNIT	PAPER NUMBER
1615	

DATE MAILED: 03/22/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Advisory Action

Application No.	Applicant(s)	
09/503,852	TILLY ET AL.	
Examiner	**Art Unit**	
Liliana Di Nola-Baron	1615	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 26 February 2004 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection. ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☒ A Notice of Appeal was filed on *26 February 2004*. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☐ The proposed amendment(s) will not be entered because:

   (a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);

   (b) ☐ they raise the issue of new matter (see Note below);

   (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

   (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____.

3. ☒ Applicant's reply has overcome the following rejection(s): 35 U.S.C. 112, first paragraph rejection.

4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☒ The a)☐ affidavit, b)☐ exhibit, or c)☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because: *See Continuation Sheet*.

6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7. ☒ For purposes of Appeal, the proposed amendment(s) a)☐ will not be entered or b)☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:

   Claim(s) allowed: _____.

   Claim(s) objected to: _____.

   Claim(s) rejected: *1,5-12,17,18,20-23,32 and 74*.

   Claim(s) withdrawn from consideration: _____.

8. ☐ The drawing correction filed on _____ is a)☐ approved or b)☐ disapproved by the Examiner.

9. ☐ Note the attached Information Disclosure Statement(s)(PTO-1449) Paper No(s). _____.

10. ☐ Other: _____

James M. Spear

JAMES M. SPEAR
PRIMARY EXAMINER
AU 1615

ontinuation of 5. does NOT place the application in condition for allowance because: Applicant's response has not overcome the 35 U.S.C. 103(a) rejection of record.

Applicant's amendment has been entered to simplify the issues for appeal. Applicant's amendment has overcome the 35 U.S.C. 112, first paragraph rejection of claims 1, 2, 4-18, 20-23, 27-36 and 46-80 of the previous Office action. Amended claims 1, 5-12, 17, 18, 20-23, 32 and 74 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perez et al. in view of Spiegel and further in view of Igarashi et al.

Perez et al. indicates that conventional cancer therapies, specifically chemotherapy, kill normal cells and one of the most sensitive noncancerous cell type is the ovarian germ cell, and teaches that apoptosis induced by the chemotherapeutic drug doxorubicin is blocked by sphingosine-1-phosphate (See e.g., p. 1228 and Abstract). Perez et al. teaches that exposure of women to a wide spectrum of agents that damage the ovary generally leads to irreversible sterility (See e.g., p. 1228) and the data from the study provide a strong impetus to manipulate apoptosis caused by chemical drugs in oocytes, in vivo, as a potential means to overcome infertility associated with cancer treatment (See e.g., p. 1231).
Perez et al. does not specify the method and dosage of administration of compositions comprising SPP.

Spiegel provides methods of retarding apoptosis in degenerative diseases, including neurodegenerative diseases and aging, by administration of sphingosine-1-phosphate and derivatives thereof (See e.g., col. 1, lines 9-17). Spiegel teaches that compositions containing SPP may be administered directly to the cells or parenterally to obtain concentrations of 0.1-100 μM, as well as to the epithelial tissues, such as the rectum and the vagina (See e.g., col. 1, line 46 to col. 2, line 42).Igarashi et al. provides methods of inhibiting tumor cell chemoinvasion, comprising administering to a host in need of treatment an inhibitory amount of sphingosine-1-phosphate and teaches that said inhibitory amount can be determined using art-recognized methods, such as dose response curves, or clinical trials, and sphingosine-1-phosphate can be administered orally, parenterally and topically, with suitable doses of sphingosine-1-phosphate depending upon the particular medical application and that the number of doses, daily dosage and course of treatment may vary from individual to individual (See e.g., col. 7, lines 32-65).

Thus, Spiegel and Igarashi et al. provide the teachings that SPP is administered in vivo and disclose a dosage for said administration. Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to apply the teachings of Perez et al. and Spiegel to device methods of protecting the female reproductive system, reviving the ovarian function or ameliorating menopausal syndromes in women, comprising administering SPP compositions, and determining the mode and dosage of administration according to the teachings of Igarashi et al. The expected result would have been successful methods of treatment. Because of the teachings of Spiegel, that sphingosine-1-phosphate is effective in treating aging diseases, and the teachings of Igarashi et al., that sphingosine-1-phosphate inhibits tumor cell chemoinvasion, one of ordinary skill in the art would have a reasonable expectation that the methods claimed in the instant application would be successful. Therefore the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made.

James M. Spear
JAMES M. SPEAR
PRIMARY EXAMINER
AU 1615

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/503,852	02/15/2000	Jonathan L. Tilly	2653/28	5439

23838 7590 12/16/2003

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EXAMINER	
DI NOLA BARON, LILIANA	
ART UNIT	**PAPER NUMBER**
1615	21

DATE MAILED: 12/16/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/503,852	TILLY ET AL.	
	Examiner	Art Unit	
	Liliana Di Nola-Baron	1615	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 September 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,4-18,20-23,27-36 and 46-80* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4-18,20-23,27-36 and 46-80* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 February 2000* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *20*.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

INFORMATION DISCLOSURE STATEMENT BY APPLICANT PTO-1449	ATTY. DOCKET NO. 2653/28	SERIAL NO. 09/503,852
	APPLICANT TILLY, et al.	
	FILING DATE February 15, 2000	GROUP 1615

U. S. PATENT DOCUMENTS

EXAMINER INITIAL	PATENT NUMBER	PATENT DATE	NAME	CLASS	SUBCLASS	FILING DATE*

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO

OTHER DOCUMENTS

EXAMINER INITIAL		AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.
LNB	1	Gong et al., "The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage", *Nature*, (1999) 399:806-809
	2	Springer et al., "Involvement of Apoptosis in 4-Vinylcyclohexene Diepoxide-Induced Ovotoxicity in Rats", *Toxicol. Appl. Pharmacol.*, (1996) 139:394-401
	3	Perez and Tilly, "Cumulus cells are required for the increased apoptotic potential in oocytes of aged mice", *Human Reproduction* (1997) 12:2781-2783
	4	Perez et al., "Prolongation of ovarian lifespan into advanced chronological age by *Bax*-deficiency", *Nature Genetics*, (1999) 21:200-203
	5	Kugu et al., "Analysis of apoptosis and expression of *bcl*-2 gene family members in th chuman and baboon ovary", *Cell Death and Differentiation*, (1998) 5:67-76
	6	Flaws et al., "Vasoactive intestinal peptide-mediated suppression of apoptosis in the Ovary: Potential Mechanisms of Action and Evidence of a conserved antiatreogenic role through evolution", *Endocrinol.* (1995) 136:4351-4359
	7	Tilly et al., "Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism", *Mol. Endocrinol.*, (1992) 6:1942-1950
	8	Johnson et al., "Susceptibility of Avian Ovarian Granulosa cells to apoptosis is dependent upon stage of follicle development and is related to endogenous lvels of *bcl*-xlong gene expression", *Endocrinol.* (1996) 137:2059-2066
	9	Greco RM. et al., "Differences in cell division and thymidine incorporation with rat and primate fibroblasts in collagen lattices", *Tissue Cell.*, (1992) 24:6 843-851

EXAMINER LNB: Nole Baio	DATE CONSIDERED 11/25/03

EXAMINER: Initial if citation considered, whether or not citation is in conformance with M.P.E.P. 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

DETAILED ACTION

Receipt of Applicant's amendment, filed on September 29, 2003, is acknowledged.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1, 2, 4-18, 20-23, 27-36 and 46-80 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). Among these factors are: (1) the nature of the invention; (2) the state of the prior art; (3) the relative skill of those in the art; (4) the predictability or unpredictability of the art; (5) the breadth of the claims; (6) the amount of direction or guidance presented; (7) the presence or absence of working examples; and (8) the quantity of experimentation necessary. When the above factors are weighed, it is the examiner's position that one skilled in the art could not practice the invention without undue experimentation.

(1) The nature of the invention:

The invention is directed to methods of protecting a female reproductive system against an artificial or natural insult, preserving, enhancing or reviving ovarian function, or preventing or ameliorating menopausal syndromes, comprising administering a composition comprising an agent that antagonizes one or more acid sphingomyelinase (ASMase) gene products.

(2) The state of the prior art

The prior art teaches that oocyte apoptosis caused by the chemotherapeutic drug doxorubicin is blocked by sphingosine-1-phosphate (SPP), an ASMase inhibitor. There is no known art wherein a certain composition is administered to successfully prevent menopausal syndrome before its occurrence, preserve or revive ovarian function, or protect the female reproductive system before the occurrence of an insult causing the undesired phenomenon.

(3) The relative skill of those in the art

The relative skill of those in the art having a Ph.D. in the biochemical or microbiological sciences, or having an M.D. is high.

(4) The predictability or unpredictability of the art

The predictability or lack thereof in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results from the claimed invention. The lower the predictability, the higher the direction and guidance that must be provided by Applicant. Menopausal syndromes, ovarian functions and an undesirable condition in the female

reproductive system can be treated, but cannot be prevented. In the instant application the predictability is very low, since menopausal syndromes, ovarian functions and unwanted conditions in the female reproductive system cannot be prevented, and consequently there is the need for a higher level of directions and guidance by Applicant. However, the amount of direction and guidance provided in the specification is limited to treatment with sphingosine-1-phosphate.

(5) The breadth of the claims

The claims are very broad. No correlation is established between the different artificial insults: chemical, radiation and surgical, nor between the various possible causes of natural insult: genetic background, physiological factors, environmental factors.

(6) The amount of direction or guidance presented

The amount of direction and guidance provided by Applicant is limited. There is no evidence in the specification that established correlation between the different artificial insults claimed by Applicant, nor, with respect to claims 22 and 23, between some of the diseases, for which the artificial insults are used as therapy. With respect to claims 62-80, no correlation has been established in the specification between the various possible causes of natural insult, which Applicant claims: genetic background, physiological factors and environmental factors.

(7) The presence or absence of working examples

The working examples present no data on the effect of the compositions of the invention on the prevention of menopausal syndromes, irregularities in ovarian functions or of an unwanted condition in the female reproductive system

(8) The quantity of experimentation necessary

The effect of the methods of the invention on the different artificial insults, for which no correlation has been established, and which are the result of therapies used for unrelated diseases, and on natural insults, which may be caused by unrelated factors, cannot be predicted a priori, but must be determined from the case to case by painstaking experimental study in vivo. When the above factors are weighed together, one of ordinary skill in the art would be burdened with undue "painstaking experimentation study" to determine a possible protecting effect of the methods claimed in the instant application.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 1, 2, 4-18, 20-23, 27-36 and 46-80 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perez et al. in view of Spiegel and further in view of Igarashi et al.

The claimed invention refers to methods of protecting female reproductive system, preserving or reviving ovarian function, or ameliorating menopausal syndromes in women, comprising administering a composition comprising sphingosine-1-phosphate (SPP).

Perez et al. indicates that conventional cancer therapies, specifically chemotherapy, kill normal cells and one of the most sensitive noncancerous cell type is the ovarian germ cell, and teaches that apoptosis induced by the chemotherapeutic drug doxorubicin is blocked by sphingosine-1-phosphate (See e.g., p. 1228 and Abstract). Perez et al. teaches that exposure of women to a wide spectrum of agents that damage the ovary generally leads to irreversible sterility (See e.g., p. 1228) and the data from the study provide a strong impetus to manipulate apoptosis caused by chemical drugs in oocytes, in vivo, as a potential means to overcome infertility associated with cancer treatment (See e.g., p. 1231).
Perez et al. does not specify the method and dosage of administration of compositions comprising SPP.

Spiegel provides methods of retarding apoptosis in degenerative diseases, including neurodegenerative diseases and aging, by administration of sphingosine-1-phosphate and derivatives thereof (See e.g., col. 1, lines 9-17). Spiegel teaches that compositions containing SPP may be administered directly to the cells or parenterally to obtain concentrations of 0.1-100

μM, as well as to the epithelial tissues, such as the rectum and the vagina (See e.g., col. 1, line 46 to col. 2, line 42).

Igarashi et al. provides methods of inhibiting tumor cell chemoinvasion, comprising administering to a host in need of treatment an inhibitory amount of sphingosine-1-phosphate and teaches that said inhibitory amount can be determined using art-recognized methods, such as dose response curves, or clinical trials, and sphingosine-1-phosphate can be administered orally, parenterally and topically, with suitable doses of sphingosine-1-phosphate depending upon the particular medical application and that the number of doses, daily dosage and course of treatment may vary from individual to individual (See e.g., col. 7, lines 32-65).

Thus, Spiegel and Igarashi et al. provide the teachings that SPP is administered in vivo and disclose a dosage for said administration. Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to apply the teachings of Perez et al. and Spiegel to device methods of protecting the female reproductive system, reviving the ovarian function or ameliorating menopausal syndromes in women, comprising administering SPP compositions, and determining the mode and dosage of administration according to the teachings of Igarashi et al. The expected result would have been successful methods of treatment. Because of the teachings of Spiegel, that sphingosine-1-phosphate is effective in treating aging diseases, and the teachings of Igarashi et al., that sphingosine-1-phosphate inhibits tumor cell chemoinvasion, one of ordinary skill in the art would have a reasonable expectation that the methods claimed in the instant application would be successful. Therefore the invention as a

whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Response to Arguments

5. Applicant's arguments filed on September 29, 2003 have been fully considered but they are not persuasive.

6. Applicant argues that apoptosis provides the correlation between the various insults and the invention provides methods of inhibiting such apoptosis. In response to said argument, it is noted that Applicant's invention is not directed to methods of inhibiting apoptosis, but to methods of protecting a female reproductive system, preventing menopausal syndromes and reviving ovarian functions. Even though apoptosis may be a common phenomenon, the diseases claimed by Applicant have different causes and need different treatment. For example, fibroids impair ovarian functions, but are not related to menopausal syndrome.

7. Applicant argues that the correlation between the insults is provided by apoptosis. In reply to said argument, it is noted that natural factors, such as genetic background, physiological factors and environmental factors, as well as artificial factors, such as drugs, act differently and do not necessarily imply apoptosis. If Applicant's invention is directed to a method of treating apoptosis, the claims should clearly read on such a method.

8. In response to Applicant's argument, that there are preventive treatments known in the art, such as vaccines, it is noted that Applicant's invention is not directed to a vaccine. Additionally, even vaccines have limited validity and several shots might be needed. Flue vaccines are good for one season only and do not provide total prevention for life. As for

Applicant's argument, that drugs control blood pressure or cholesterol level, said drugs have a controlling effect, not a preventing effect, and do not prevent heart attacks. Applicant has not provided any guidance in the specification that administration of the drug prevents a disease before its occurrence. Since said guidance is not present in the specification, undue experimentation would be necessary. It is recommended to amend the claims of the instant application to read on methods of treatment, rather than preventing or preserving.

9. Applicant argues that Perez et al. discloses studies, which were performed in vitro and contains specific statements of doubt as to whether the results obtained in vitro can be extrapolated to in vivo treatment of the female reproductive system, and Spiegel and Igarashi et al. are not directed to the field of Applicant's invention. In response to said arguments, it is noted that the statement in Perez et al. that "These data provide a strong impetus for our current efforts to manipulate death effector pathways in oocytes, in vivo, as a potential means to overcome infertility associated with cancer treatment" (See p. 1231), strongly suggests to apply the treatment in vivo. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to administer the composition in vivo for the treatment of infertility associated with cancer therapy. The examiner relies on Spiegel and Igarashi et al. for their teachings that SPP is administered in vivo and the disclosure of a dosage for said administration. With respect to the field of Applicant's invention, Spiegel teaches the use of sphingosine-1-phosphate (SPP) to retard apoptosis in degenerative diseases, including aging. Additionally, Spiegel teaches that SPP may be administered to the epithelial tissues, such as the rectum and the vagina (See e.g., Col. 1, line 46 to col. 2, line 26). Igarashi et al. provides the teachings that sphingosine-1-phosphate can be administered orally, parenterally and topically (See e.g., col. 7,

lines32-65). Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to combine the teachings of Perez et al., Spiegel and Igarashi et al. to device methods of protecting the female reproductive system, reviving the ovarian function or ameliorating menopausal syndromes in women, comprising administering SPP compositions, and determining the mode and dosage of administration according to the teachings of the prior art The expected result would have been a successful method of protecting a female reproductive system against natural or artificial insults.

Conclusion

10. Claims 1, 2, 4-18, 20-23, 27-36 and 46-80 are rejected.

11. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Liliana Di Nola-Baron whose telephone number is 703-308-8318. The examiner can normally be reached on Monday through Thursday, 5:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Thurman K Page can be reached on 703-308-2927. The fax phone number for the organization where this application or proceeding is assigned is 703-305-3592.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 308-1234/ 1235.

LDN03

December 2, 2003

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	JONATHAN L. TILLY et al.
SERIAL NO.	:	09/503,852
FILING DATE	:	February 15, 2000
FOR	:	PROTECTION OF THE FEMALE REPRODUCTIVE SYSTEM FROM NATURAL AND ARTIFICIAL INSULTS
EXAMINER	:	L. DI NOLA-BARON
GROUP ART UNIT:		1615
CONFIRMATION NO.:		5439

COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelop addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 2231 1450 on

Date: February 24, 2004

By: _____
Joseph A. Coppola (Reg. No. 38,413)

AMENDMENT UNDER 37 C.F.R. §1.116

Sir:

In response to the Office Action dated December 16, 2003, please consider the following amendments and remarks intended to simplify the issues for appeal. Enclosed herewith is a Notice of Appeal.

CLAIM AMENDMENTS

This listing of claims replaces all prior versions and listings of claims in the application:

1. (currently amended) A method of ~~protecting~~ treating a female reproductive system ~~against an artificial insult comprising:~~ by administering to a female patient a composition comprising ~~an agent that antagonizes one or more acid sphingomyelinase (*ASMase*) gene products,~~ sphingosine-1-phosphate in an amount sufficient to ~~protect said female reproductive system from destruction caused by said~~ inhibit apoptosis induced by an artificial insult, wherein said administration is *in vivo* or *ex vivo*, and wherein said artificial insult is a chemotherapeutic drug or radiation.

2-4. canceled

5. (currently amended) The method of ~~claim 4~~ claim 1, wherein said chemotherapeutic drug ~~comprises;~~ is 5FU, vinblastine, actinomycin D, etoposide, cisplatin, methotrexate, doxorubicin, or a combination thereof.

6. (currently amended) The method of claim ~~2~~ 1, wherein said radiation insult ~~comprises~~ is ionization radiation, x-ray, infrared radiation, ultrasound radiation, heat, or a combination thereof.

7. (currently amended) The method of claim ~~2~~ 1, wherein said radiation insult comprises an invasive radiation therapy, a non-invasive radiation therapy, or both.

8. (original) The method of claim 1, wherein said female reproductive system comprises ovaries.

9. (original) The method of claim 1, wherein said female reproductive system comprises oocytes.

10. (previously presented) The method of claim 1, wherein said female patient is in a reproductive age.

11. (previously presented) The method of claim 1, wherein said female patient is in a pre-reproductive age.

12. (previously presented) The method of claim 1, wherein said female patient is in a post-reproductive age.

13-16. canceled

17. (original) The method of claim 1, wherein said composition is administered at least once from about fifteen days to about two days prior to exposure to said insult.

18. (original) The method of claim 17, wherein said composition is administered at about seven days to about two hours prior to exposure to said insult.

19. canceled

20. (original) The method of claim 1, wherein said composition is administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly, intra-uterine, intra-ovarian, rectally, topically, or a combination thereof.

21. (original) The method of claim 1, wherein said artificial insult is a result of a therapy against a disease or a disorder.

22. (original) The method of claim 21, wherein said disease or disorder comprises, cancer, rheumatoid arthritis, angioplasty, or restenosis.

23. (original) The method of claim 22, wherein said cancer comprises; colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chondroma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma,

embryonal carcinoma, Wilms' tumor, cervical cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, acute lymphocytic leukemia and acute myelocytic leukemia, chronic leukemia and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain diseases, or a combination thereof.

24-31. canceled

32. (currently amended) The method of claim ~~27~~ 1, wherein said ~~mammal~~ female patient is a woman.

33-73. canceled

74. (currently amended) The method of claim 1, wherein the artificial insult is ~~a chemical insult and the chemical insult is~~ a chemotherapeutic drug.

75-80. canceled

Remarks

Before this Amendment, claims 1, 2, 4-18, 20-23, 27-36, and 46-80 were pending. This Amendment cancels claims 2, 4, 13-16, 27-31, 33-36, 46-73, and 75-80. If this Amendment is entered, claims 1, 5-12, 17, 18, 20-23, 32, and 74 will be pending.

Claim 1 has been amended to recite inhibiting apoptosis. Support for this amendment is found in the specification at page 9, lines 6-13 and page 17, lines 2-6. The other amendments to claim 1 merely incorporate limitations found in dependent claims. Thus, these limitations also are supported.

The amendments to claims 5, 6, 7, 32, and 74 relate either to matters of form, changes in dependency, or the incorporation of a limitation from another claim.

The rejections under 35 U.S.C. §112

The Applicants do not agree with the rejections under 35 U.S.C. §112, but have nevertheless amended claim 1 in an effort to simplify the issues for appeal and thus advance the prosecution of certain subject matter within prior claim 1. Claim 1 has been amended to take into account comments made by the Examiner in the Office Action dated December 16, 2003 in connection with the rejections under 35 U.S.C. §112. It is hoped that this Amendment will be entered, the rejections under 35 U.S.C. §112 will be withdrawn in view of the amendments made to claim 1, and the appeal will proceed limited to the issues raised by the obviousness rejection.

Claim 1 has been amended to recite "treating" in view of the Examiner's comments at pages 8-9, paragraph 8, of the Office Action.

Claim 1 has been amended to recite "sphingosine-1-phosphate" in view of the Examiner's comments in the last sentence of the first paragraph of page 4 of the Office Action.

Claim 1 has been amended to recite "inhibit apoptosis" in view of the Examiner's comments at pages 8, paragraphs 6-7, of the Office Action.

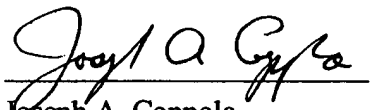
Claim 1 has been amended to recite "wherein said artificial insult is a chemotherapeutic drug or radiation" since the Applicants have submitted evidence that chemotherapeutic drugs act via causing apoptosis and the specification contains a working example of radiation treatment.

In view of these amendments to claim 1, it is respectfully requested that the rejections under 35 U.S.C. §112 be withdrawn and the application proceed to appeal with the set of claims listed in this Amendment.

The time for responding to the Office Action was set for March 16, 2004. Therefore it is believed that this Amendment is timely and no Petition for the Extension of Time is required. If this is in error, please treat this Amendment as containing a Petition for the Extension of Time under 37 C.F.R. § 1.136(a) for a period sufficient to permit the filing of this Amendment. Charge any corresponding fees to Kenyon & Kenyon's Deposit Account No. 11-0600.

The Applicants hereby also make a Conditional Petition for any relief available to correct any defect seen in connection with this filing, or any defect seen to be remaining in this application after this filing. The Commissioner is authorized to charge Kenyon & Kenyon's Deposit Account No. 11-0600 for the Petition fee and any other fees required to effect this Conditional Petition.

Respectfully submitted,

Dated: February 24, 2004 By: 

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